

A SIMPLIFIED DETERMINATION OF REDUCTANT-SOLUBLE PHOSPHATE IN SOIL PHOSPHATE FRACTIONATION SCHEMES¹

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Abstract

A simplified and more rapid procedure for the determination of reductant-soluble phosphorus (P) was developed by a modification of the Murphy and Riley (1962) ascorbic acid method. The procedure which eliminated the interference of sodium dithionite by air-oxidation and that of sodium citrate by addition of ammonium molybdate, was suitable for the determination of 0.1 to 0.7 μg P/ml in the presence of up to 3000 μg Fe/ml and 4.0 μg Si/ml.

Additional Index Words: citrate interference in phosphate determinations.

THE DETERMINATION OF reductant soluble P mobilized by citrate-bicarbonate-dithionite (CBD) extraction (Chang and Jackson, 1957) is subject to potential interferences from dithionite, citrate, iron, and silicon. Although there are presently colorimetric procedures that circumvent these interferences (Chang, Chu, and Erh, 1966; Petersen and Corey, 1966), they are tedious and some of them involve the use of hazardous reagents. A more straightforward method would be a definite improvement. It is the purpose of this Note to present a modification of an ascorbic acid method for P (Murphy and Riley, 1962) that allows a simplified, direct determination of P in CBD extracts.

Materials and Methods

REAGENTS

Reductant Soluble P—The reagents for the extraction of reductant soluble P are those of Chang and Jackson (1957).

Phosphorus Determination—The reagents required for the phosphorus determination by the ascorbic acid method of Murphy and Riley (1962) are those described by Watanabe and Olsen (1965). One additional reagent that is necessary is a 5% ammonium molybdate solution and this is prepared by dissolving 50 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 1 liter of distilled water. The solution should be stored in a polyethylene bottle.

PROCEDURE

Oxidize the sodium dithionite that may be remaining in the reductant soluble P extracts obtained by either the Chang and Jackson (1957) or Petersen and Corey (1966) procedure by

Table 1—Influence of MoO_4^{2-} concentration on development of molybdophosphoric acid in the presence of citrate

Watanabe and Olsen (1965)*	Molybdate (MoO_4^{2-})		Citrate	P	Absorbance†		
	Added	Total			15 min	60 min	12 hours
	mmoles			μg			
0.71	0	0.171	0	20	0.240	0.238	0.241
0.71	0	0.171	0.610	20	0.000	0.000	0.000
0.171	0.283	0.454	0.610	20	0.000	0.000	0.012
0.171	0.586	0.757	0.610	20	0.057	0.187	0.239
0.171	0.849	1.02	0.610	20	0.239	0.241	0.240
0.171	1.13	1.30	0.610	20	0.241	0.238	0.242
0.171	1.42	1.59	0.610	20	0.262	0.300	0.346
0.171	0.849	1.02	0.610	5	0.059	0.090	0.090
0.171	0.849	1.02	0.610	25	0.300	0.298	0.302
0.171	0.849	1.02	0.610	35	0.422	0.420	0.421

* 0.171 mmole MoO_4^{2-} is that amount present in 5-ml aliquot of the Watanabe and Olsen (1965) P reagent.

† Measured at 750 m μ with a Bausch and Lomb Spectronic 20 Colorimeter.

bubbling moist air through the extracts for 4 hours. Pipette 5-ml aliquots from the air-oxidized extracts into 50-ml volumetric flasks. Next, pipette 3 ml of the 5% ammonium molybdate solution into each flask. Add distilled water to make approximately a 40-ml volume and add 5 ml of reagent B of Watanabe and Olsen (1965). Add distilled water to make the flasks to volume; mix thoroughly and measure the absorbance at 750 m μ after 30 min.

The color is stable up to 4 hours. If higher sensitivity is necessary, the absorbance measurements can be made at 882 m μ . For the standard curve, it is necessary that an air-oxidized CBD solution blank be prepared. If the Chang and Jackson extraction procedure for reductant-soluble P is used, the CBD solution blank is prepared by mixing 40 ml of 0.3M sodium citrate, 5 ml of 1M sodium bicarbonate, 50 ml of saturated sodium chloride, 1 g of sodium dithionite, and diluting to 100 ml with distilled water. If the Petersen and Corey procedure is used, mix 25 ml of 0.3M sodium citrate, 1 g sodium dithionite, and dilute to 50 ml with distilled water. After bubbling moist air through the solution blanks for 4 hours to oxidize the sodium dithionite, pipette 5-ml aliquots of the appropriate CBD solution blank into 50-ml volumetric flasks. Pipette aliquots of a 5 μg /ml standard P solution to give a range from 0 to 35 μg of P and proceed as given above for the unknowns.

Results and Discussion

The air-bubbling step, as in the determination of Si in CBD extracts (Weaver, Syers, and Jackson, 1968), was found to be an effective and efficient means of oxidation of excess dithionite in the CBD extracts. Failure to carry out the oxidation of excess dithionite results in the precipitation of elemental sulfur upon addition of the acid-molybdate reagent.

The addition of the 5% ammonium molybdate solution was found to be necessary to compensate for the interference from citrate. The latter causes interference by virtue of the fact that citrate and molybdate form a stable complex on a 1:1 molar basis (Killefer and Linz, 1952). Therefore, when the concentration of citrate exceeds that of molybdate, molybdophosphoric acid complex formation fails by virtue of the fact that all of the molybdate is complexed with citrate. It can be clearly seen in Table 1 that the interference from citrate was readily overcome when the amount of added molybdate was sufficiently in excess of citrate. For this purpose, the most suitable amount of molybdate to add was found to be 0.849 mmole or 3 ml of 5% ammonium molybdate solution. This amount was adequate for rapid full color development of the reduced molybdophosphoric acid over the range of 5 to 35 μg P, and

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Table 2—Recovery of added P in CBD extracts of three Puerto Rican soils

Soil series and subgroup	In Aliquot		P			Recovery of that added
	Fe	Si	In Aliquot	Added	Recovered	
			μg			%
Daguerre Orthoxic Tropohumult	2,230	57	8.0	20.0	28.0	100
Los Guineos Epiaque Humoxic Tropohumult	1,890	47	6.9	20.0	27.2	101
Pfina Typic Haplorthox	645	14	3.0	20.0	23.6	103

did not cause self reduction of molybdate which occurred when the amount of added molybdate was 1.42 nmole.

The proposed procedure was found to be tolerant to a wide range of Fe and Si concentrations. Added Fe ranging from 0.5 to 1.5 mg Fe/50 ml showed no interference through the range of 5 to 35 μg P. With the sample size and dilutions used in the Chang and Jackson procedure, 1.5 mg Fe in the 5-ml test aliquot would correspond to 43% Fe_2O_3 , an amount of CBD extractable Fe that will be encountered only in extremely weathered soils or ferruginous crusts. The presence of up to 75 μg Si/50 ml was found to have no adverse effect on P determination by the proposed procedure. At 100 μg Si/50 ml, a slight positive interference of approximately 10% was noted. With the sample size and dilutions used, however, 100 μg Si in the 5-ml test aliquot would correspond to 2.0 mg Si/g soil, an amount higher than that encountered in most CBD soil extracts (Weaver et al., 1968). Therefore, the slight interference of Si should not be a serious limiting factor.

As a final test of the proposed procedure, it was applied to the recovery of added P in CBD extracts of three Puerto Rican soils. The amounts of Fe and Si in the extracts were determined colorimetrically by 1, 10 phenanthroline (Jackson, 1956) and reduced molybdosilic acid (Weaver et al., 1968), respectively. The results (Table 2) indicated a satisfactory recovery of added P (100–103%).

Literature Cited

1. Chang, S. C., W. K. Chu, and K. T. Erh. 1966. Determination of reductant-soluble phosphate in soils. *Soil Sci.* 102: 44–45.
2. Chang, S. C., and M. L. Jackson. 1957. Fractionation of soil phosphorus. *Soil Sci.* 84:133–144.
3. Jackson, M. L. 1956. *Soil chemical analysis—Advanced course* (sixth printing). Published by the author, Dep. of Soil Science, Univ. of Wisconsin, Madison, Wis. 53706.
4. Killefer, D. H., and A. Linz. 1952. Molybdenum compounds: Their chemistry and technology. Interscience Publishers, New York. p. 90–92.
5. Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31–36.
6. Petersen, G. W., and R. B. Corey. 1966. A modified Chang and Jackson procedure for outline fractionation of inorganic soil phosphate. *Soil Sci. Soc. Amer. Proc.* 30:563–565.
7. Watanabe, F. S., and S. R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO_3 extracts from soil. *Soil Sci. Soc. Amer. Proc.* 29:677–678.
8. Weaver, R. M., J. K. Syers, and M. L. Jackson. 1968. Determination of silica in citrate-bicarbonate-dithionite extracts of soil. *Soil Sci. Soc. Amer. Proc.* 32:497–501.